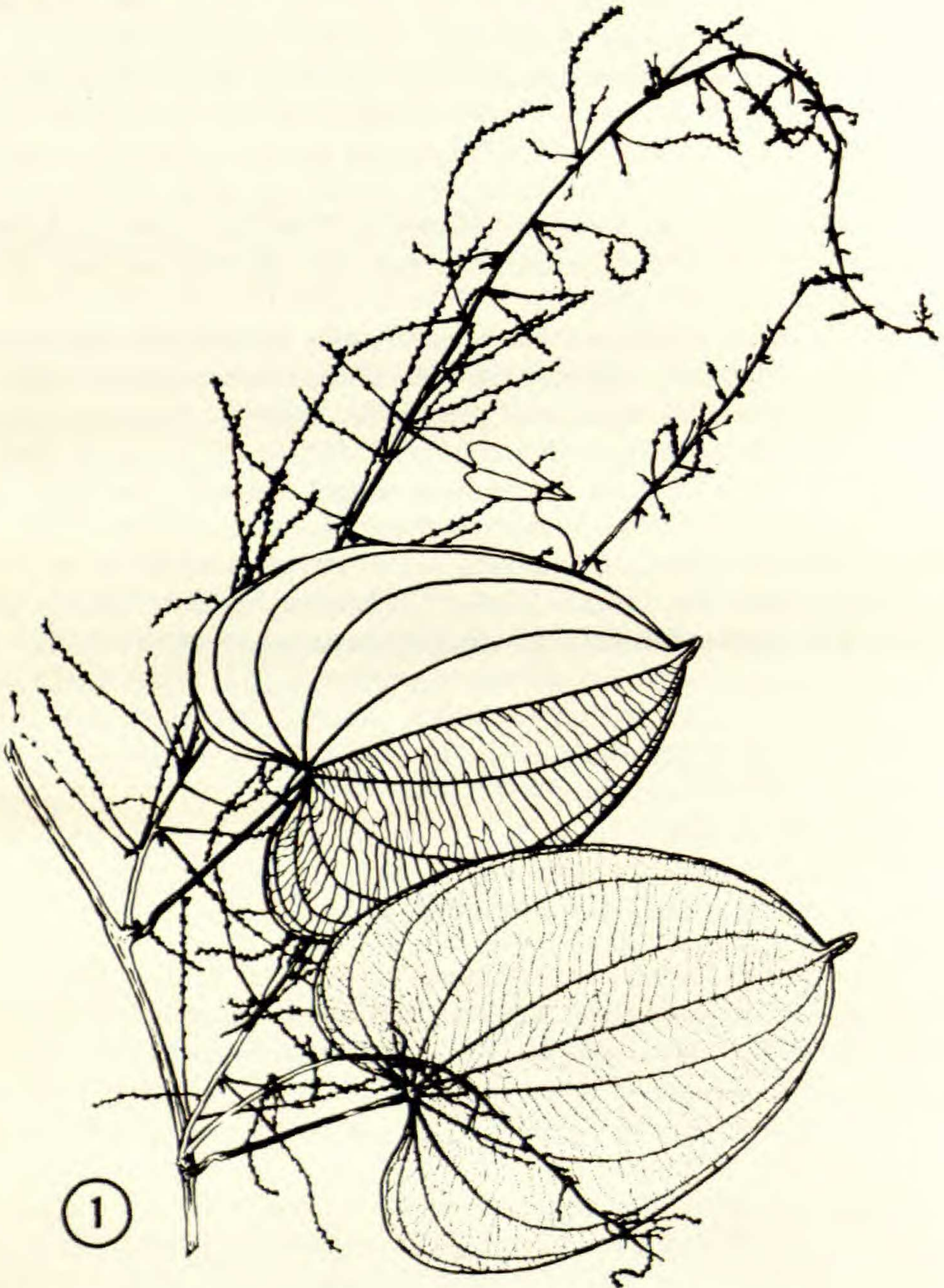


## ANALYSIS OF THE COMPLEX VASCULARITY IN STEMS OF *DIOSCOREA COMPOSITA*

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PLANT ANATOMISTS HAVE, for many years, been presented with various diagrams designed to represent the course of vascular bundles in monocotyledons. In a comprehensive study Bary (1884) classified the vas-



FIGURES 1 and 2. Habit of *Dioscorea composita* Hemsl.,  $\times 1/2$ . 1. Male inflorescence.



cular systems of the monocotyledons into three main categories viz., the palm type, the type of the Commelinaceae, and that of the "anomalous monocotyledons," i.e. those "which differ fundamentally from . . . the very great majority of Monocotyledons."

In spite of some variation in the vascular tissue in stems of the palm type, the salient features of this construction are similar. Leaf trace bundles are present in all stems together with main vascular stem bundles. The main vascular bundles have the tendency to run near the periphery of the stem axis and nearly parallel with it. A large number of bundles from each leaf enter the stem separately and take a radially oblique course. In large plants such as the palms the number of bundles reaches several hundred. The bundles from different parts of the same leaf penetrate



2. Female inflorescence.

2



the stem to different depths. The midvein penetrates deepest, while those of the margins extend the least distance.

The stem type of the Commelinaceae was described as similar to the dicotyledonous arrangement of tissues. The stem bundles occur in a circle lying near the periphery. These are some distance from the leaf trace bundles which are near the center.

The so-called anomalous type of stem, including that of the Dioscoreaceae, was described as nearest to that of the dicotyledons in its vascular system. The leaf traces are arranged in a single circle around the circumference of the central cylinder. The vascular bundles penetrate radially into the stem center, extending to unequal depths.

In an early study of the stem of the Dioscoreaceae Mohl (1845) made a comparison with the stem of the Palmae. He concluded that the vascular architecture of these two families was similar although there were some peculiar histological details in the Dioscoreaceae.

Zimmermann and Tomlinson, in a series of articles on the anatomy of the palm *Rhapis excelsa* and *Prionium* (Juncaceae) (Zimmermann & Tomlinson, 1965, 1967, 1968; Tomlinson & Zimmermann, 1966a, 1966b, 1968a, 1968b), have made some very revealing studies showing more accurately than had previously been done the pathway of the vascular bundles. The studies of *Rhapis excelsa*, *inter alia*, are intended to serve as a model to represent (a) the course of vascular bundles including (1) leaf trace; (2) vertical bundles; (3) bridge bundles and (4) satellite bundles; (b) the dynamic flexibility that enables some monocotyledons to produce variable numbers of vascular bundles; and (c) the fact that there is no continuity of metaxylem between the stem and the leaf, hence, the leaf is irrigated solely by protoxylem.

In a recent publication (Ayensu, 1969), an attempt, based on the conventional technique, was made to summarize and explain how the vascular tissue of two successive internodes maintains continuity in the complex nodal structure in stems of the Dioscoreaceae. It became evident that a more reliable understanding of the pathway in the vascular system would be gained by the use of the motion picture analysis technique.

Four species of the Dioscoreaceae (*Dioscorea composita* Hemsl., *D. polygonoides* Humb. & Bonpl., *D. friedrichsthali* R. Knuth and *Tamus communis* L.) have so far been analysed by the Optical Shuttle method. *Dioscorea composita* has been selected to illustrate here the pathways observed in the species listed.

#### MATERIALS AND METHODS

*Dioscorea composita* (FIGURES 1, 2) is a vigorously growing climber with a glabrous, cylindrical, woody stem. The vine has a tendency to become sulcate when dry. The leaves are alternate, simple, and with entire margins. The leaf blade is coriaceous, broadly ovate, cordate at base, abruptly acute at apex, or sometimes with the apex obtuse or apiculate.



The leaves are 10–25 cm. long and 5–20 cm. wide with 7–9 veins prominent on both surfaces. Petioles are 6–12 cm. long, sulcate and rather firm. The male inflorescence is prominent, 1–2 in the axils, compound, 20–30 cm. long, and with rachis slightly tomentose. Flowers lack pedicels, are 1–2–3-agglomerate, and occur about 2–3 mm. apart. Bracts are acute, and broadly lanceolate. The perianth is fleshy, tubular, slightly tomentose, and about 3 mm. long; its segments are ovate-orbicular, obtuse and erect, and almost the same length as the tube. The fertile stamens are 6, central, and erect; the filaments averaging 2 mm. long are unequal and fleshy. The style is rudimentary but conspicuous. The female inflorescence is elongate, simple or compound; the capsules are ovoid-oblong, 1.5–2.5 cm. long. The tubers are large, long, and penetrate deeply into the soil.

From the seeds sent me by Dr. Franklin W. Martin, plant geneticist, U.S. Department of Agriculture, Mayaguez, Puerto Rico, I was able to obtain germinated seeds of *Dioscorea composita* in petri dishes at 80° F, in ten days. Between two and three months later the vines were harvested for histological studies. Other material used for this study was collected by me during a visit to Puerto Rico. A herbarium specimen of *D. composita* (Baldwin, 14238), collected in Vera Cruz, was also revived (cf. Ayensu, 1967) and used for this study.

Material previously stored in the fixative FAA was washed thoroughly in running water for one hour. The stem pieces were then removed from the running water and passed through a series of alcohol concentrations from 50 percent to absolute. From the absolute alcohol the stem pieces were processed through the following changes:

- |  |                                    |
|--|------------------------------------|
| 1) 2/3 abs. alcohol and 1/3 chloroform | 5) 2/3 chloroform and 1/3 paraffin |
| 2) 1/2 abs. alcohol and 1/2 chloroform | 6) 1/2 chloroform and 1/2 paraffin |
| 3) 1/3 abs. alcohol and 2/3 chloroform | 7) 1/3 chloroform and 2/3 paraffin |
| 4) 2 changes of pure chloroform        | 8) 2 changes of paraplast          |

These alcohol-chloroform-paraffin/paraplast changes were conducted in an oven at intervals of 30 minutes.

Paraplast blocks containing the stems were cut at 10 $\mu$ , on a rotary microtome. Each section was cut and mounted on a separate slide. Sections were numbered sequentially to facilitate the cinematographic analysis. The sections were dewaxed, stained with a mixture of safranin (95 parts of 1 percent solution in 70 percent ethanol) and Delafield's haematoxylin (5 parts), dehydrated, and mounted in Canada balsam.

The serial analysis of the sections was accomplished with the aid of the optical shuttle system (cf. Zimmermann & Tomlinson, 1965, 1966, 1968) consisting of two identical microscopes, discussion and image overlap tube (Zimmermann Adapter), and a 16 mm. movie camera. A Data Analyser Projector was used in the study of the films.

## OBSERVATIONS

**Anatomy of mature aërial stem.** The paragraph which follows is a



summary of the description of the internode encountered in *Dioscorea composita*.

Trichomes absent; glands and stomata present. Cuticle thick and ridged. Epidermis composed of thick-walled circular or dome-shaped cells with cytoplasmic contents. Cortex of 6 to 10 layers of cells of variable size; 1-3 outer layers collenchymatous. Endodermoid layer separating cortex from 2 to 3 layers of fibers. Both common and cauline vascular bundles present. Common bundles V-shaped with one phloem unit at converging ends of the V, and two phloem units terminating the flanges of the V. Cauline bundles elliptical with one pair of large vessels each of average diameter of  $430\mu$ , and with one large phloem unit on inner side of innermost pair of large vessels close to the center of the pith, and another phloem unit at the outer end. Average diameter of large sieve tubes  $96\mu$ . Pith area constituting one fourth of central cylinder. Crystals: idioblast-containing raphide bundles with cuboidal crystals are also present. Tannin cells observed. Extra floral glands present.

**Course of the vascular bundles.** Internodes from the middle portion of the plant show the following progression: (1) Starting from the base upwards in an internode, it is observed that 8 common and 8 cauline vascular bundles maintain their strict individuality, as well as their shape, until about  $850\mu$  before a connection is made with the nodal region. At this point alteration occurs in the two, or in some special cases in the four, cauline vascular bundles facing the direction of the leaf insertion. The large phloem units on the inner side of the innermost pairs of metaxylem vessels begin to break down and anastomose (FIGURES 4, 5). (2) About  $1550\mu$  from above the innermost protoxylem elements of the same cauline bundles begin to anastomose. (3) This is followed by the fusion of the phloem elements from the two or four cauline bundles into a full manifestation of a phloem glomerulus. (4) Next is the development of the xylem glomerulus (FIGURE 6). (5) Following (3) and (4), the phloem units and the xylem elements of the common vascular bundles begin to anastomose into xylem and phloem glomeruli (FIGURE 7). (6) The large metaxylem vessels, until now not affected by the structural changes in the cauline vascular bundles, begin to lose their identity by merging into the xylem glomerulus (FIGURES 8-10). (7) The more centrally located phloem glomerulus "moves" radially between the remnants of the two adjacent cauline bundles and joins the outer one towards the glomerulus of the common bundle organized in the same radial direction. (8) The glomeruli of the other common bundles "move" in the direction of the point of the petiole insertion during the movement of (7). (9) The xylem glomerulus formed within the cauline bundles "follows" rapidly behind the phloem glomerulus described in (8), thereby completing the breakdown and the incorporation of the large metaxylem vessels into the plexus. (10) The phloem units at the outer end of the cauline vascular bundles are unaffected by the processes of anastomosis occurring in the node. Instead, the position of the phloem unit is altered only slightly by the stresses applied as a result of the outward "movement" of the xylem and phloem glomeruli. (11) At about the time that the outward "movement" of the glomeruli is completed, the reorganization of the tissues of the inner side of the cauline bundles begins. This reorganization is rapidly followed by the return of the outer half of the cauline bundle into its original position. At this point the elliptical shape of the bundles observed in transverse sections at the internode and in the petiole is restored (FIGURES 11, 12). (12) The reorganization of the common bundles follows the process described for (11). (13) At  $12,480\mu$  above the node



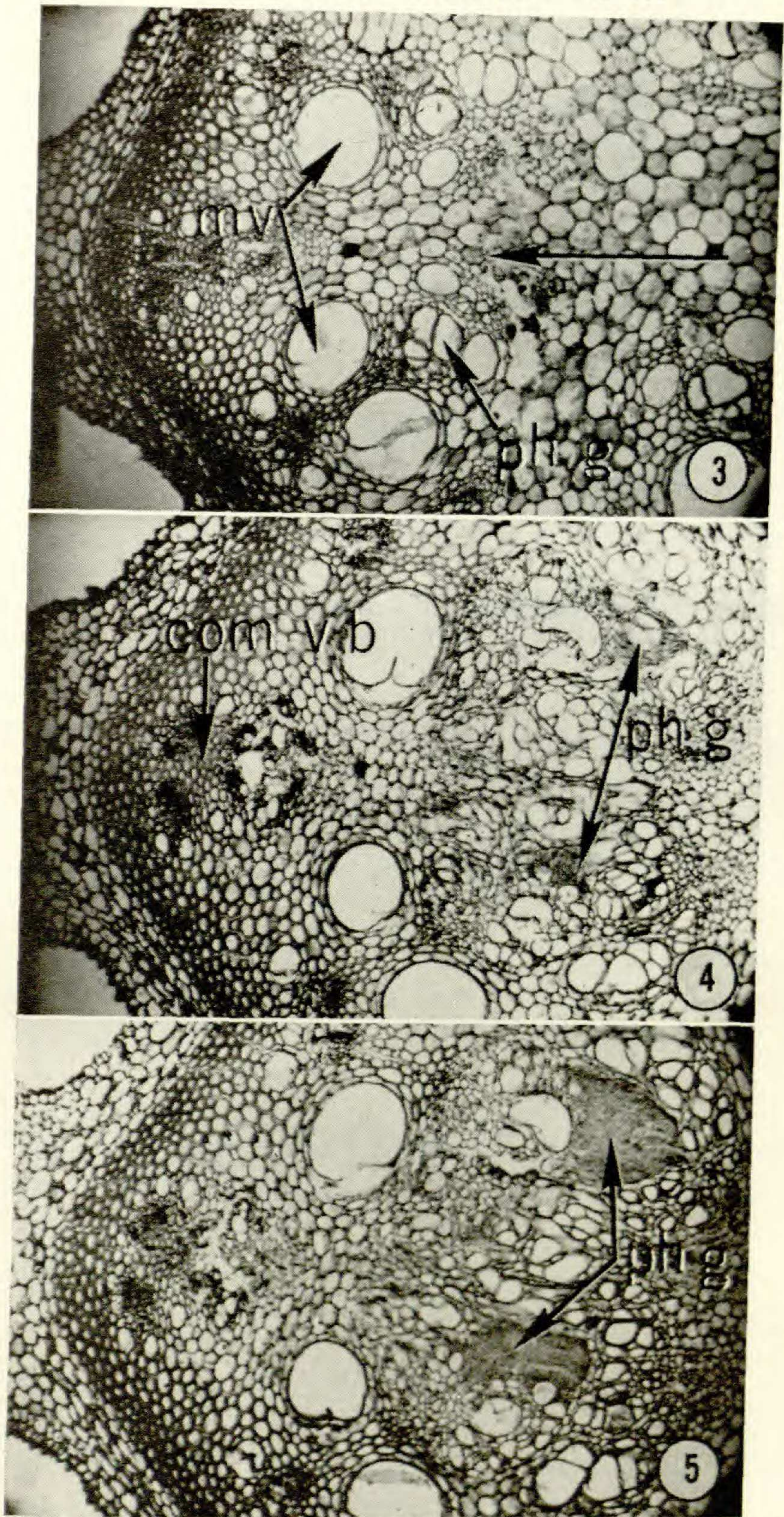
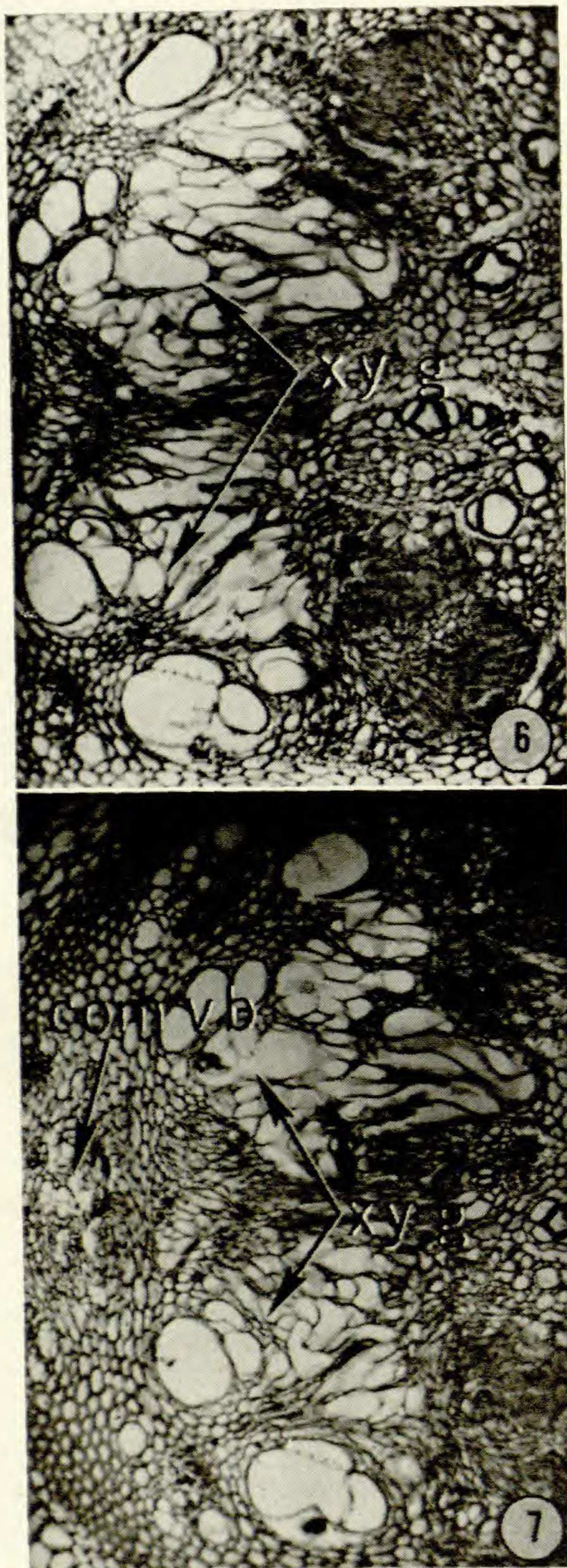


FIGURE 3. Internodal region where each vascular bundle maintains its individuality,  $\times 105$ . Center arrow indicates direction of leaf insertion. Distance between FIGURES 3 and 4 is  $380\mu$ .

FIGURES 4 and 5. Large phloem units on inner side of the innermost pair of metaxylem vessels begin to break down and anastomose,  $\times 105$ . Distance between FIGURES 4 and 5 is  $150\mu$ .

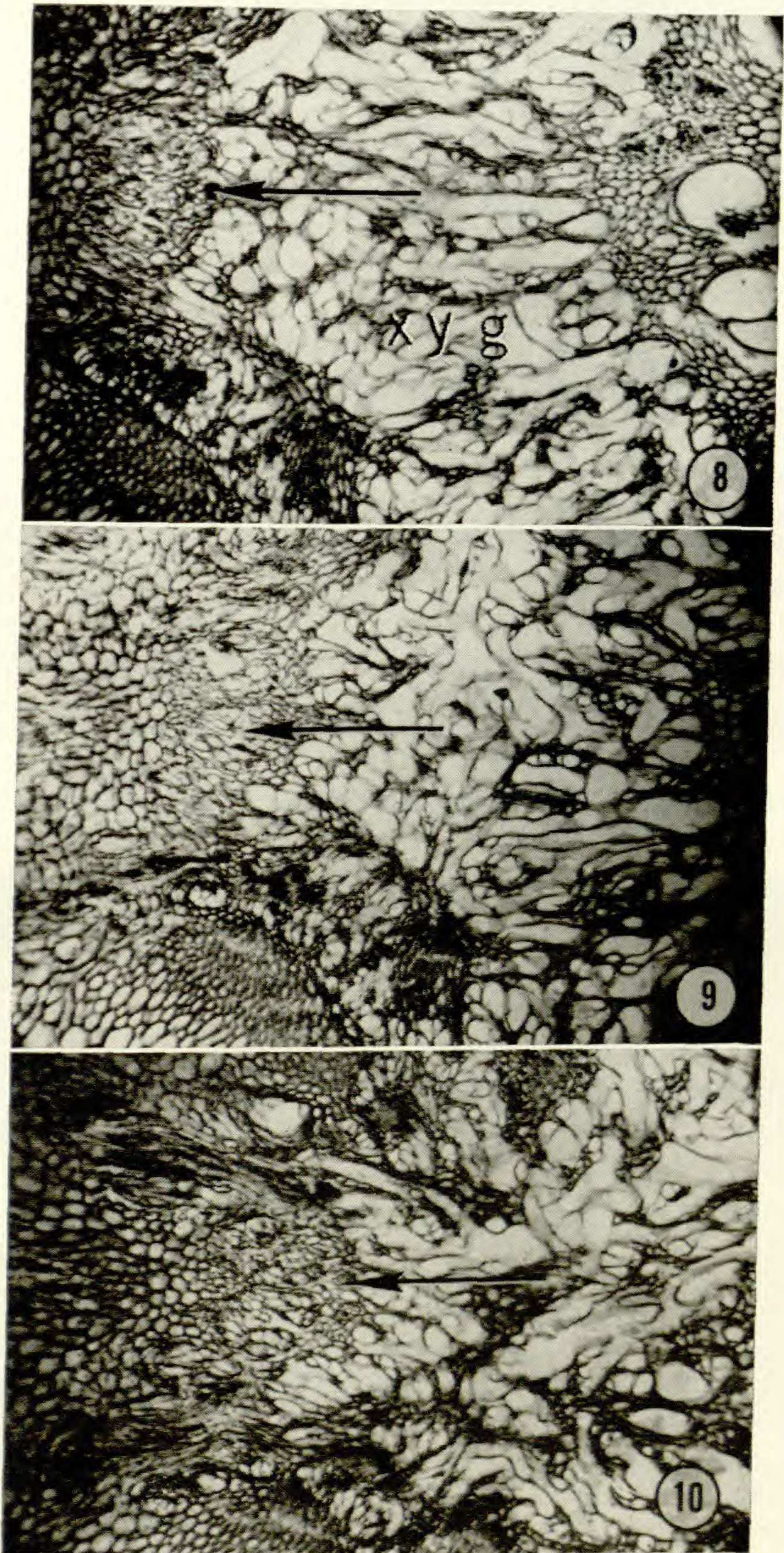




FIGURES 6 and 7. Large metaxylem vessels begin to lose their identity,  $\times$  105. Distance between FIGURES 6 and 7 is  $80\mu$ .

ABBREVIATIONS (for these and the figures which follow): ph. g., phloem glomerulus; xy. g., xylem glomerulus; com. v.b., common vascular bundle; cau. v.b., cauline vascular bundle; m. v., metaxylem vessel; ph. u., phloem unit; m. s., main stem; p., petiole; r. b., raphide bundles.





FIGURES 8-10. Sequence showing the "movement" of phloem and xylem glomeruli in the direction (arrow) of the point of the petiole insertion,  $\times 105$ . Distance between FIGURES 8 and 9 is  $20\mu$  and between FIGURES 9 and 10 is  $30\mu$ .



the reorganization is complete and internodal structure with usually 8 common and 8 cauline bundles is entirely restored.

### DISCUSSION

Although the number of bundles varies between species, and the alternation of common and cauline bundles is an established fact, it has been observed that occasionally two common bundles "run" side by side. This irregularity may be explained as follows: At the node two cauline bundles originate in the plexus and take their positions in the spaces on either side of the middle common bundle and the lateral common bundles. This ratio of two cauline and three common bundles is corrected a little farther down the stem by the elimination in the plexus of one of the lateral common bundles. Therefore, in the internode there are almost always one or more common bundles in excess of the one to one ratio of common and cauline bundles. This irregularity is detected only in the internode because the common bundles never unite during their course in the internode. In a similar manner the cauline bundles of some specimens of *Dioscorea macroura*, *D. preussii*, and *D. sansibarensis* sometimes split into two with all the vascular elements above the pair of innermost metaxylem vessels forming a separate unit radially above the metaxylem.

**Comparison of Dioscorea with Rhapis.** In the course of this study it became quite obvious that the vascular architecture of *Dioscorea composita* was strikingly different from that of *Rhapis excelsa* (Palmae) described as a model for the monocotyledons by Zimmermann and Tomlinson (1965). Their detailed study of the vascular bundles revealed four types of branch systems. A leaf trace was found to originate by the gradual transformation of the vertical bundle which reaches its maximum size towards the center of the stem and turns sharply towards the stem periphery where it breaks up into several branches. The metaxylem deficient bundle that enters the leaf as a leaf trace was found to be the direct continuation of the vertical bundle. Zimmermann and Tomlinson (1965) concluded that there was no continuity of metaxylem between stem and leaf. Hence the leaf was irrigated solely by protoxylem. Apart from the studies on *Rhapis excelsa* the origin of the leaf trace and the discontinuity of the metaxylem into the leaf has also been demonstrated in other monocotyledonous genera including *Washingtonia*, *Geonoma* (Palmae), and *Prionium* (Juncaceae). The present study, however, has demonstrated that in *Dioscorea composita* (and in other species of Dioscoreaceae) the leaf is irrigated not solely by the protoxylem but by the metaxylem as well (FIGURES 6-8).

Zimmermann and Tomlinson (1965) also noted that in most cases a single vertical bundle arises from a trace plexus. However, they observed that in many cases two or more bundles may arise. The implication of this irregularity in the number of vertical bundles is that a certain measure of flexibility exists in the palms and in other monocotyledons which mani-



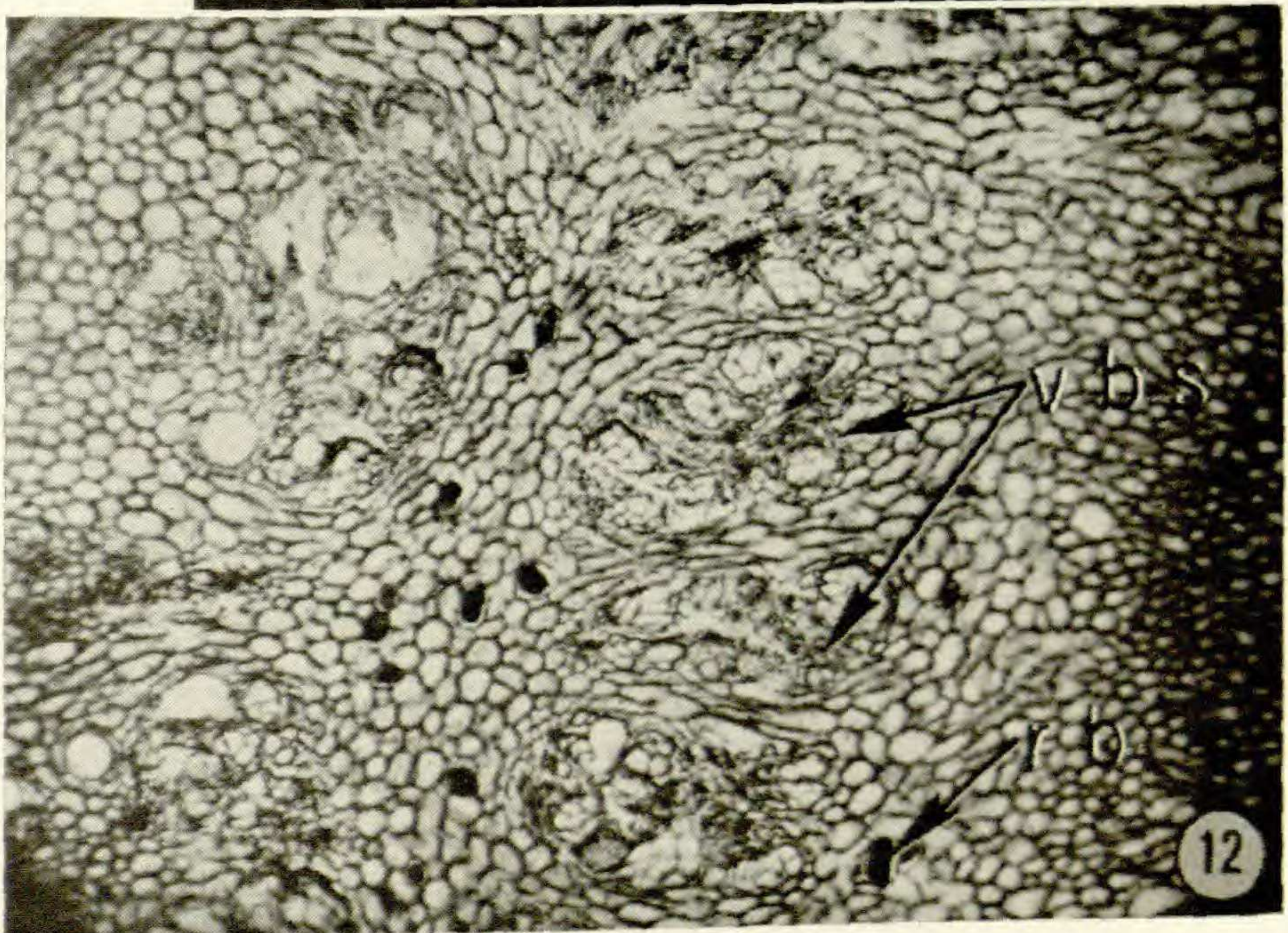
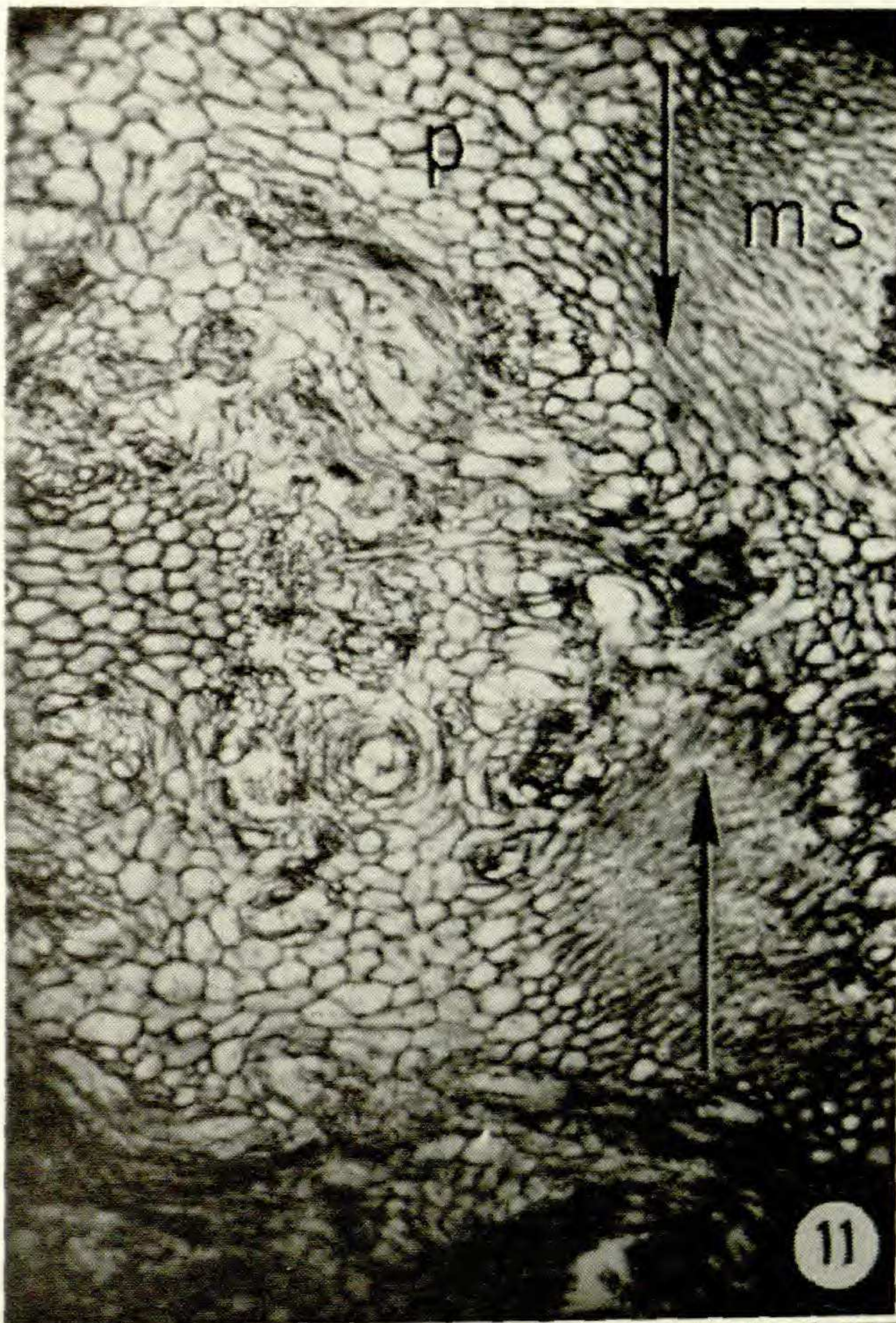


FIGURE 11. Arrows indicating the junction between the main stem and the petiole,  $\times 105$ .

FIGURE 12. Transverse section of the petiole showing the reorganization of the vascular bundles in the petiole,  $\times 105$ . Distance between FIGURES 11 and 12 is  $40\mu$ .



festis itself in the unequal, sometimes large number of bundles observed in stems.

The Dioscoreaceae exhibit remarkable constancy in the total number of bundles in the stem. With few exceptions, the number of cauline and common vascular bundles established early in ontogeny is maintained in the plant, regardless of the formation of xylem and phloem glomeruli that reduce all the vascular bundles entering the nodal region into a plexus.

The bridge bundles described and demonstrated in cinematographic analysis as branches from each departing leaf trace in *Rhapis* and *Prionium* were not encountered in *Dioscorea composita*. Bridges are short (the longest one measured in *Rhapis* is 4.5 mm.) and they make a leaf trace and a vertical bundle appear to fuse directly. If we were to assume that bridge bundles were indeed present in the Dioscoreaceae they must emerge from the leaf trace together with the vertical bundles in a single package.

Satellite bundles which Zimmermann and Tomlinson (1965) described as vascular branches from larger leaf traces originating in the region of bridge production were not observed in *Dioscorea composita*. Satellite bundles are best observed in plants which have fully developed inflorescences. Zimmermann and Tomlinson also stated that even in plants where inflorescences have aborted very early in their development and are not exomorphically visible, satellite bundles can still be detected.

These comparisons drawn between the Palmae and the Dioscoreaceae after using similar instrumentation and methods confirm the fact that the vascular systems of these two taxa are basically different. This study also contradicts the earlier belief (cf. Falkenberg, 1876; Mohl, 1845) that the Palmae and the Dioscoreaceae do share a common architectural framework.

The use of the Palmae as a model for the representation of the course of vascular bundles in the stems of monocotyledons is being questioned by some plant anatomists in view of the uniqueness of the palms amongst the monocotyledons. Vascular pattern studies in such genera as *Tradescantia* (Scott & Priestly, 1925), *Alstroemeria* (Priestly, Scott, & Gillett, 1935), *Ananas* (Krauss, 1948), *Oryza* (Majumdar & Saha, 1957), *Zea* (Kumazawa, 1961), *Ripogonum* (Simpson & Philipson, 1969) and my own unpublished data on the bamboo *Shibutaea kumusaca* and *Smilax megalantha* have shown conclusively that because of distinct variations which exist it is too early yet to consider the structure of any one taxon as a model for the Monocotyledoneae.

#### SUMMARY

The complex vasculature of the aërial stems of *Dioscorea composita* was traced through the use of the Optical Shuttle method and the Data Analyser Projector. This cinematographic analysis has shown that the course of vascular bundles in *D. composita* and the other species of Dioscoreaceae so far examined, differs markedly from that of the palm



*Rhapis excelsa*. Furthermore, the commonly held view that the Dioscoreaceae and the Palmae share a common vascular framework is not supported. In view of the variations that exist in the vascular patterns in a number of monocotyledons, it is suggested that the selection of any one taxon as a typical monocotyledon should await results of further studies.

#### ACKNOWLEDGMENTS

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